RESPONSES OF PLANTS TO HORMONE-LIKE SUBSTANCES

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My assignment concerns the work at the Boyce Thompson Institute with plant hormones and chemical compounds which induce a hormone-like response. The program committee was kind enough to have my paper preceded by one from Dr. Avery dealing with the historical phases of the subject. I will limit myself, therefore, to the work at the Institute. What I have to say involves also the work of two of my associates, Drs. A. E. Hitchcock and Frank Wilcoxon, plant physiologist and chemist, respectively. I wish to fully recognize the part they have taken in the work, though I shall not attempt to point out individual contributions. Dr. William Crocker, Director of the Institute, has also maintained a very active interest in our work on growth-promoting chemicals, especially as related to unsaturated carbon gases.

In recent years several terms have been used to designate growth-promoting substances (plant hormones, growth hormones, growth substances, growth regulators, auxins, etc.). As used in this paper, the following definitions will apply: *Plant hormones* are substances, produced by the plant and which exert a regulatory influence on growth and development of the organism. As far as green tissues are concerned, these "substances" have been shown to exist but not one has been chemically identified. Hormones are present in minute amounts and must not be confused with materials having a direct nutritive effect. *Natural growth substances* carries the same meaning as "hormone." *Auxin*, or hetero-auxin, is used to designate "Auxin A" or "B" or indoleacetic acid, which were extracted from urine, corn meal, etc. (but not green tissue) and assigned chemical formulae by Kögl. They are therefore classed with other known chemical compounds which induce hormone-like responses in plants. *Synthetic growth substances* are any chemical compounds which, when applied locally or otherwise, induce hormone-like responses in plants. They include compounds that cause bending, swelling, proliferations, and overgrowths of stems and leaves, and in some cases the induction of adventitious roots. As to form, they may be acids, salts, esters, or gases.

During the past few years, 32 growth-promoting substances have been reported. Among them are 14 acids, 11 esters, and four unsaturated carbon containing gases. Also salts of the various acids are effective.

The most important substances from a scientific standpoint are naphthaleneacetic acid, indoleacetic acid, indolebutyric acid, indolepropionic acid, fluoreneacetic acid, phenylacetic acid, ethylene, propylene, acetylene, and carbon monoxide. For practical plant propagation, naphthaleneacetic acid and indolebutyric acid are the most important.

The tomato plant has been extensively used as a test object when effective chemicals were being compared or when new compounds were tested for activity. Many other species, however, serve the purpose as well as tomato. Details of the tests will be mentioned later.

A simple method for applying substances locally is to mix a given amount of the chemical with a known quantity of lanolin (wool fat) and apply the preparation with a glass rod to the part of the plant to be treated. A one per cent mixture is usually convenient at the start. If this is found too strong (as would be the case with naphthaleneacetic acid) it can be readily diluted with pure lanolin. Other carriers, as paraffin oil, olive oil, etc., might be found more useful than lanolin for some purposes.

Water solutions of growth substances are effective over a wide range of concentrations from 1 part in 5,000 to 1 part in 1,000,000. The dilutions must be varied according to the substances and the uses to which they are put.

In 1933 three papers (9, 10, 11) reported that four unsaturated gases (carbon monoxide, ethylene, acetylene, and propylene) induced epinasty (due to accelerated growth on the upper side of the petiole) and initiated adventitious roots on stems and leaves of many species of plants. These responses involved cell enlargement, cell division, and the organization of cells for the production of adventitious organs. The gases were applied to the entire plant or to a single root or leaf with the result that the chemical moved into the tissue and spread throughout the plant, causing a systemic response (Fig. 1A). The gases were also dissolved in water and applied in solution with similar effects.

Figure 1. Tomato plants. A., Left, control; middle, treated with one part of ethylene gas to 500,000 parts of air. Photographed 24 hours later. Right, treated on the upper side of the petioles with a lanolin preparation containing 0.1 per cent naphthaleneacetic acid. B., Left, control; right, cut surface treated with 1.0 per cent naphthaleneacetic acid. Photograph taken eight days later.

Beginning in 1934 the responses induced by the simple compounds (gases) were compared with responses induced by more complex substances, ring compounds (2). Though administered in a different way, the end results were about the same for both groups of chemicals. The crystalline compounds have the advantage of being easily applied in water, oil, or lanolin, inducing either local or systemic responses.

The following are some of the responses to be considered: Local acceleration of growth causing bending of stems and leaves (epinasty); systemic responses vs. local; swelling and proliferation of treated tissue; cell division and induction of adventitious roots; acceleration of growth following retardation; and retardation of root elongation followed by increase in diameter and induction of adventitious roots.

When lanolin preparations of growth substances are applied to the upper side of a tomato petiole, growth is accelerated locally and the result is downward movement of the leaf (Fig. 1A). The degree of movement varies with the concentration of the substance and the age or condition of the leaf.

Similarly, if a growing stem is treated on one side with a low concentration, the growth rate is increased and the stem is caused to bend away from the treated side (negative bending). If the concentration is too high, growth is retarded and the stem bends toward the treated side (positive bending).

Local application of very low concentrations induces only a local response, but if high concentrations are used the substances are translocated and tend to induce a systemic response as shown by epinasty of leaves beyond the treated region. As with gas-treated plants, the upper side of the petioles respond first and, therefore, the downward growth of the leaf. The systemic effect is readily induced by injecting a water solution into the tissue or by watering the soil with a solution of the substance. To inject the substances it is well to use a glass tube holding 0.3 cc. and drawn to a capillary at one end. Insert the capillary into the stem and allow the contents to drain into the tissue. The time required varies with the plant, the weather conditions, and the time of day. Usually two to four hours are sufficient if the plant is in a light, dry, place. For this purpose use approximately 0.1 mg. of the substance per cc. of water. When the solution is added to the soil, use 10 to 40 mg. in 50 cc. of water, depending upon the activity of the substance and the size of the plant. An 8-inch high tomato plant in a 4-inch pot will respond when 20 mg. of indolebutyric acid in 50 cc. of water are used. Half of that concentration is sufficient for naphthalemeacetic acid or indoleacetic acid.

Bending responses can be detected within one to two hours after the substance is applied. The activity continues and the response is complete within six to ten hours. Within 48 hours another kind of activity, swelling of the tissue, becomes evident. This phase of the response is associated with cell division and the activity may continue until the stem breaks open or ruptures the epidermis. Proliferations and gall-like growth occur when some kinds of plants are used. These are sometimes compared with crown galls or tumors. They differ, however, in that they do not continue to grow long after the substance is removed. Crown galls continue to grow as long as living organisms are present, but malignant tumors of animals are not so regulated. The tumor seems to provide its own stimulus once initiated.

A third type of response becomes evident within five to ten days when adventitious roots begin to make their appearance. Stems, leaves, flower stalks, and young fruit can all be induced to form roots while the plant is intact. If the top of a growing plant is removed and the chemical applied to the cut surface, a crown of roots soon appears (Fig. 1B).

There is considerable variation in the root-inducing power of the growth substances applied to intact plants, naphthaleneacetic acid being the most effective and the phenyl compounds among the poorest. The latter often cause much swelling with few or no roots while indolebutyric acid may cause little swelling and many roots.

There is an important difference between the response of stem tissue and that of roots. Cells of growing stems can be increased in length by treatment with growth substances. That is the cause of bending when the chemical is applied unilaterally. Cells of root tissue on the other hand not only do not increase in length when treated, but are actually retarded by the substance. When aerial roots of Cissus are treated with comparable concentrations along one side with only a trace of growth substance, positive bending results. If the tip is treated the entire region of elongation is retarded. In other respects stems and roots respond very much alike. Following treatment there is evident swelling and increase in diameter and then within three to six days numerous branch roots appear. The same substances have been used to induce adventitious roots on roots and roots on stems. This raises the question whether there might be a group of chemicals, not now recognized, which have the power to induce shoots or other organs of plants.

Mention was made of the absorption of growth substances from the soil. The rate at which the chemicals were taken up the stem was detected by stem bending and epinasty of the leaves. There was evident variations with weather conditions as with transpiration and it was assumed, therefore, that with this method of application the substance traveled in the transpiration stream. If the rate of transpiration was reduced by increasing relative humidity or placing plants in the dark, the rate of movement of the substance from the roots was also reduced.

However, responses induced by lanolin preparations applied to the aerial parts were not influenced by atmospheric conditions in the same way. Darkness or high relative humidity favored absorption from lanolin preparations through the epidermis and longitudinal movement in both directions. Apparently, therefore, movement of these substances occurs also outside the transpiration stream.

In this connection it should be pointed out that growth substances can be absorbed from water solutions through the tips of the leaves. If the tip of a tomato leaf attached near the center of an eight-inch plant is placed in a solution of naphthaleneacetic acid (10 to 30 mg. per 100 cc. of water) or indoleacetic acid, epinasty of the treated leaf occurs within an hour or two. This response is soon followed by bending of the stem and epinasty of other leaves, both below and above the treated leaf. The movement downward, however, is more rapid than upward. When treating leaves in this way it was found best to break the epidermal hairs or otherwise damage the tissue to facilitate entrance of the chemical.

CONDITIONS AND FACTORS AFFECTING THE RESPONSE OF PLANTS TO GROWTH SUBSTANCES

It was evident from the first that varying the environmental conditions changed the capacity of plants to respond when treated with any of the several growth substances. Plants in different stages of maturity, or similar plants at different seasons of the year varied in their capacity to respond to treatment. Sunlight and complete darkness brought out some facts which should be considered.

A large number of six-inch tomato plants in four-inch pots were placed in the dark room with a temperature of 75° to 80°, and each day for a week several of the plants were subjected to a series of tests. The tests consisted of treatment of leaves and stems with lanolin preparations of growth substances; subjecting the plants to an atmosphere containing ethylene gas; placing plants in a horizontal position to induce the negative geotropic response (bending away from the earth).

The summarized results are as follows: The plants lost their capacity to respond (epinasty of leaves) to ethylene gas in three days, and to the growth substances in six to seven days. When placed in a horizontal position the plants failed to right themselves after the fourth day (Fig. 2A). At this time, however, the horizontal plants could be induced to turn upward if treated on the lower side of the stem with lanolin preparations of the growth substances (Fig. 2B).

Geotropic responses are assumed to be due to a redistribution of natural hormones in favor of the lower side (8). If this theory is correct, plants losing their capacity to grow upward presumably have lost all their natural hormones. Plants in this condition should make good test objects to determine which chemical compounds act like hormones and might therefore properly be called "growth substances." On that basis all of the naphthalene, indole, and phenyl compounds mentioned at the beginning of this paper could qualify (3).

It appears that growth responses involve a chain of essential compounds. If any one is missing there can be no growth. The plants in dark for several days lose their natural hormone (1) which has to do with geotropic response and, therefore, cannot right themselves when placed in a horizontal position. The growth substances have been substituted for the "missing link." It could be further assumed that if any essential elements, for example sugar, were absent there could be no growth until a satisfactory substitute was supplied. Perhaps the substitute could be another sugar different from the natural product.

There was some relation between the food storage organs and the length of time plants could tolerate darkness. Large tomato and tobacco plants lasted much longer than small ones. Plants with storage organs—dahlias, artichokes and potatoes—grown in the dark from the first, kept their capacity to respond to gravity and treatment with growth substances for 60 to 80 days (Fig. 2c).

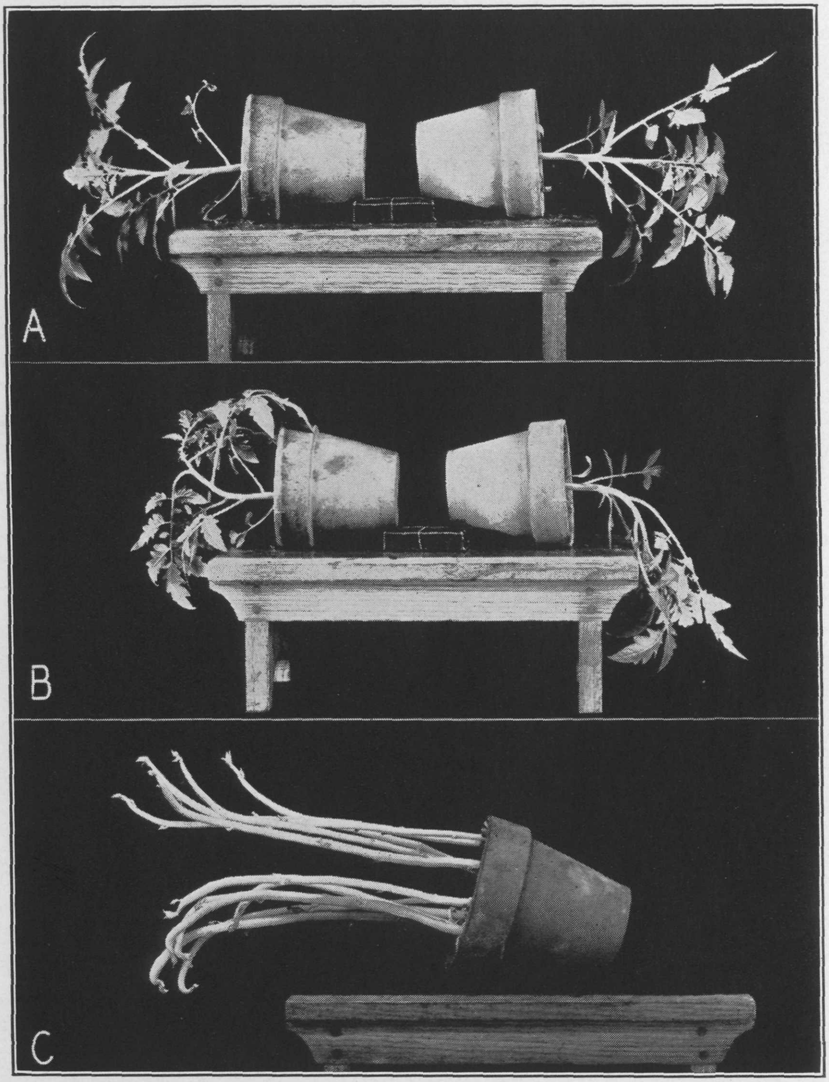

Figure 2. A., Tomato plants in the dark four days, then placed in a horizontal position and photographed 24 hours later to show loss of capacity to respond to force of gravitation. B., Same plants as in A. 24 hours after treatment with 1.0 per cent indolepropionic acid. Left, treated on under side; right, treated on upper side. C., Potato plants grown in dark for 25 days, then placed in horizontal position, at which time lower six shoots treated on upper side with naphthaleneacetic acid. Upper shoots left for controls show negative geotropism.

It appears, therefore, that light is not essential for the production of natural hormones in growing tissue if storage organs are present. On the other hand, small seedling plants (tomato and tobacco) soon lost their natural hormones if kept in continuous darkness. The rate at which small seedlings lost their hormones varied with the temperature. Plants kept at 50° F. for 10 days still responded to gravity ethylene gas and other growth substances while those at 80° lost their capacity to respond after four to six days. Transferred back to light these plants regained their capacity to respond to gravity and growth substances.

It is not clear from these data what part light plays in producing hormones in seedlings in contrast with the supply in plants having storage organs and grown in the dark.

PRACTICAL APPLICATIONS

The botanical laboratories will find many uses for growth substances.

In the search for natural hormones and synthetic compounds which induce hormone-like effects on plants, several different methods have been developed. The *Avena* method which employs the oat coleoptile as the test object is well known. This has been considered by some investigators as the standard method. To obtain reliable results with this procedure, it is necessary to use a standardized strain of oats, to maintain a constant temperature in a dark room with high humidity, and to become highly skilled in handling the equipment, conducting the experiments, and collecting the data. In fact the difficulties involved discourage all but the most persistent investigators. Another objection to the *Avena* test is that it measures only one of several growth responses induced by hormone-like substances—namely, the capacity of a substance to cause cell elongation. To satisfy all the requirements, a method should be capable of measuring cell enlargement, cell division, penetration, and the induction of new organs. At the present time no one method has been found that can fulfill all of these requirements.

The technique developed in the laboratory at the Boyce Thompson Institute makes use of the tomato plant though many other species can be satisfactorily used. No special skill is required. The chemical being investigated is mixed with lanolin or olive oil and applied to one side of the stem at the region of elongation and on the upper side of the adjacent petiole. If the substance is active the leaf bends downward and the stem bends away from the leaf thus increasing the angle between the treated parts. This response occurs within one to six hours. After a compound is found effective, dilutions are made and compared with a known standard which is usually α-naphthaleneacetic acid. A concentration of one part of this substance to 1,000,000 parts of lanolin will induce epinasty of tomato leaves.

The same technique serves also to test the root-inducing capacity of a substance for intact plants but higher concentrations are required. The highly active substances induce roots over a range of concentrations beginning with 0.001 per cent and going up to the toxic limit which is usually around 1.0 to 3.0 per cent.

The capacity of a substance to spread throughout the plant and thus induce systemic responses is determined with the use of lanolin preparations and water solutions. The first application of lanolin preparations serves for studying induced acceleration of growth causing bending from cell enlargement, cell division, penetration, systemic response, and production of adventitious roots. Responses involving retardation and various types of proliferation can also be determined. The water solutions are added to the soil of potted plants, injected into stems and leaves, and used for tests involving immersion of leaves. Also leafy cuttings of tomato are convenient test objects for quick response. If the basal end of a six-inch cutting is placed in a solution of an effective substance, induced epinasty of leaves becomes evident within an hour or two.

Plant propagation. To test the root-inducing capacity of substances to be used in connection with propagation of plants from cuttings, *Ligustrum* and *Euonymus* are generally used. They are usually available and both hardwood and summer cuttings respond readily. The basal ends of the cuttings are placed in water solutions of the substances for 24 hours and then planted in a rooting medium. The controls under this condition start rooting in 20 days. If the substances are effective the treated cuttings will have developed roots in 14 days.

The most important substance for propagating plants by cuttings is indolebutyric acid. Naphthaleneacetic is second and indoleacetic acid third in importance. The indolebutyric is effective over a wider range of concentrations than the other substances.

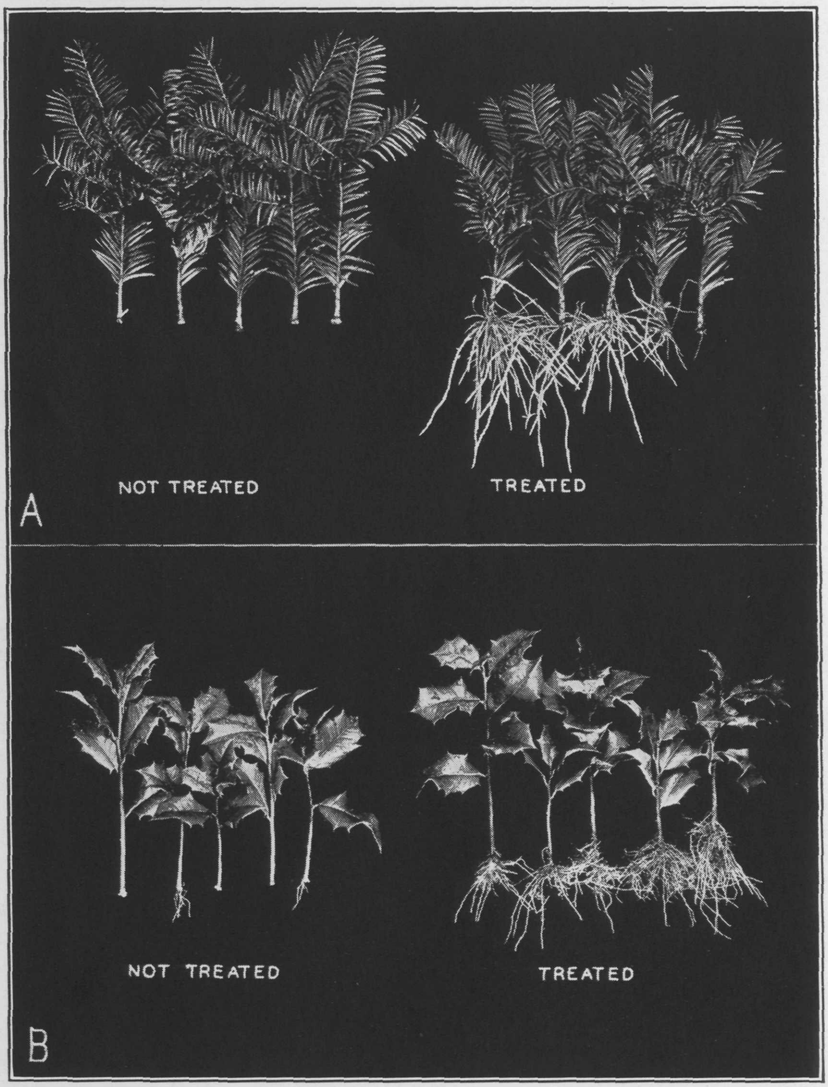

Figure 3. Cuttings treated with water solution of indolebutyric acid for 24 hours, then planted in the rooting medium. A., Taxus. B., Holly.

With the aid of this substance we have been able to hasten the rooting of species which are ordinarily propagated by cuttings and to induce roots on cuttings of species which will not root otherwise. An adequate root system can be quickly formed and the resulting plants readily established. Some of the difficulties encountered in the past with plants from cuttings concerned a meager root system. This phase of the problem can now be eliminated.

The question is often asked, do these chemically treated cuttings produce normal roots? The only answer I can make is that the resulting root system appears normal and after being planted in soil the new plant is readily established.

Approximately eighty-five genera, involving several hundred species and varieties, have been found to make satisfactory response when given the chemical treatment. Taxus and holly (Fig. 3) can be rooted in half the time required ordinarily for control cuttings. Both young and old wood work equally well. Roses and dogwood are among the most sensitive types responding in a very short time to low concentrations of the substance. Apples and pears vary with the variety. They do not respond when hardwood cuttings are used but several varieties can be propagated readily from treated greenwood cuttings. In general, leafy cuttings of all species respond better than leafless, hardwood types. The difficulty may be that the leafless cuttings do not take up the material from solution. However, it has been encouraging to find that many species respond when both hardwood and green cuttings are used.

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